Ver 1.0

HB1060

2010

■ CELL SV MAXI
CLINIC SV MIDI/MAXI
BLOOD SV MIDI/MAXI

exgene™

DNA PURIFICATION HANDBOOK

GeneAll

Customer & Technical Support

Do not hesitate to ask us any question.

We thank you for any comment or advice.

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This protocol handbook is included in :

GeneAll® Exgene™ Blood SV Midi (105-226,105-201)
GeneAll® Exgene™ Blood SV MAXI (105-310, 105-326)
GeneAll® Exgene™ Clinic SV Midi (108-226, 108-201)
GeneAll® Exgene™ Clinic SV MAXI (108-310, 108-326)
GeneAll® Exgene™ Cell SV MAXI (106-310, 106-326)

Visit www.geneall.com or www.geneall.co.kr for FAQ, QnA and more information.

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	Blood SV				Clinic SV				Cell SV	
Cat. No.	**105-226**	**105-201**	**105-310**	**105-326**	**108-226**	**108-201**	**108-310**	**108-326**	**106-310**	**106-326**
Size	**Midi**	**Midi**	**MAXI**	**MAXI**	**Midi**	**Midi**	**MAXI**	**MAXI**	**MAXI**	**MAXI**
Preps.#	26	100	10	26	26	100	10	26	10	26
Columns	26	100	10	26	26	100	10	26	10	26
Tubes	52	200	20	52	52	200	20	52	20	52
Buffer GP (ml)	-	-	-	-	-	-	-	-	60	150
Buffer YL (ml)	-	-	-	-	-	-	-	-	60	150
Buffer CL (ml)	-	-	-	-	60	200	60	200	60	200
Buffer BL (ml)	80	300	150	320	80	300	150	320	150	320
Buffer BW (ml)	90	400	90	220	90	400	90	220	90	220
Buffer TW (ml)	100	500	120	360	100	500	120	360	120	360
Buffer AE (ml)	30	120	60	120	30	120	60	120	60	120
Proteinase K (mg)	60	240	48	120	60	240	48	120	48	120
PK Storage bfr. (ml)	4	14	4	7	4	14	4	7	4	7
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Precautions and disclaimer

GeneAll® Exgene™ Midi and MAXI series are for research use only, and should not be used for drug, household or other unintended uses. All due care and attention should be taken in every procedure in this handbook. Please consult the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Storage Condition

All components of GeneAll® Exgene™ Midi and MAXI series should be stored at room temperature (15~25°C). After reconstitution of Proteinase K with storage buffer, it should be stored under 4°C for conservation of activity. It can be stored at 4°C for 1 year without significant decrease in activity. But for prolonged preservation of activity, storing under -20°C is recommended.

Under cool ambient condition, a precipitate can be formed in buffer CL and/or BL. In such a case, heat the bottle above 37°C to dissolve completely. GeneAll® Exgene™ Midi and MAXI series are guaranteed until the expiration date printed on the product label.

Chemical Hazard

Buffer BL and BW contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions.

GeneAll® Exgene™
Blood/Clinic/Cell SV Midi/MAXI

Introduction

GeneAll® Exgene™ Midi and MAXI series provide fast and easy methods of large scale purification of total DNA from various biological samples, such as whole blood, cultured cells, tissues, bacteria and etc.

GeneAll® Exgene™ Series utilize the advanced silica-binding technology to purify total DNA sufficiently pure for many applications. Various samples are lysed in optimized buffer containing detergents and lytic enzyme. Under high salt condition, DNA in the lysate bind to silica membrane and impurities pass through membrane into a collecting tube. The membranes are washed with a series of alcohol-containing buffer to remove any traces of proteins, cellular debris and salts. Finally pure DNA is released into a clean collection tube with deionized water or low ionic strength buffer.

Typical yield

Sample	Amount	Typical Yield
Human Whole blood	2 ml	20 ~ 60 ug
Animal tissue	100 mg	20 ~ 80 ug
Cultured cells	2×10^7 cells	40 ~ 100 ug
Bacteria cells	1×10^{10} cells	30 ~ 100 ug
Yeast	5×10^8 cells	70 ~ 200 ug

General Information

- All centrifugation on procedures MUST be performed on a tabletop centrifuge which has a swinging bucket. Using fixed-angle-rotor centrifuge will cause poor yields of DNA recovery. Refer to next page for suitable centrifuge.

- Centrifugal force should be over 4,000 xg at least to get proper result.
  Low centrifugal force will cause the incomplete washing and eluting, followed by poor result.

- Unless RNase A is treated, both DNA and RNA will be co-purified.
  RNA may inhibit some downstream enzymatic reactions, but not PCR itself. If RNA-free DNA is required, RNase should be treated before addition of Buffer BL.

- The size of purified DNA is up to 50kb in length, and the majority is 20 ~ 30 kb approximately. This fragments will be completely denatured during PCR and it can be amplified with high efficiency.

- DNA purified by GeneAll® Exgene™ Midi and MAXI procedure is free of protein and other contaminants which may inhibit PCR or other enzymatic reactions. Purified DNA can be applied to various downstream applications, such as PCR, Southern blotting, RAPD, AFLP, RFLP and other subsequent enzymatic reactions. DNA can be used immediately or safely stored in buffer AE at -20°C for later use.

Using Swinging-bucket Centrifuge in Midi/MAXI procedures

GeneAll® Exgene™ Midi and MAXI procedures require the conventional centrifuge which has a swinging-bucket rotor and ability of 4,000 ~ 5,000 xg. Use of fixed-angle rotor will cause nonuniform contact between column membrane and solutions, followed by inconsistent result. Low g-force will lead to uncomplete removal of ethanol from column membrane and to inadequate eluting. Compatible centrifuges and rotors are listed below, but you can use any other equivalent.

Company	Centrifuge	Rotor
Beckman CoulterInc. (California, USA)	Allegra X-15R Allegra 25R	Sx4750 Sx4750A TS-5.1-500
Eppendorf AG (Hamburg, Germany)	5804/5804R 5810/5810R	A-4-44
EYELA Inc. (Tokyo, Japan)	5800 5900	RS-410 RS-410M
Hanil Science Industrial Inc. (Incheon, Korea)	Union 5KR Union 55R MF-550 HA1000-6 HA1000-3	R-WS1000-6B W-WS750-6B HSR-4S WHSR-4S
Hettich AG (Kirchlengern, Germany)	Rotina 35 Rotanta 460 Rotixa 50S	1717 1724 5624

GeneAll® Exgene™ Protocols

Cell SV MAXI

Clinic SV Midi/MAXI

Blood SV Midi/MAXI

GeneAll

A PROTOCOL for 0.4 ~ 1 ml of whole blood

Blood SV Midi

Clinic SV Midi

Before experiment

Prepare the water bath to 56°C

Prepare absolute ethanol

If a precipitate has formed in buffer BL, heat to dissolve completely

1. **Pipet 50 ul of Proteinase K solution (20 mg/ml, provided) into the bottom of a 15 ml centrifugation tube (not provided).**

2. **Add 1 ml of the sample to the tube and mix well.**
   If the sample volume is less than 1 ml, bring the volume of sample to 1 ml with PBS.

3. **Add 1.2 ml of Buffer BL to the tube. Vortex the tube for 15 sec to mix thoroughly.**
   For efficient lysis and consistent result, it is essential to mix the sample completely.

4. **Incubate at 56°C for 20 min.**
   During incubation, occasional vortexing of the lysate will help accelerate lysis. Longer incubation will not affect DNA recovery.

5. **Add 1 ml of absolute ethanol (not provided) to the sample, vortex to mix the sample thoroughly.**
   It is essential to mix the sample completely for efficient binding.

6. **Transfer all of the mixture to a Midi column carefully, close the cap, centrifuge for 3 min at 2,000 xg (3,000 rpm).**
   While transfer of the mixture to a Midi column, be careful not to moisten the rim of a Midi column.
   If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

7. **Discard the filtrate and re-insert the Midi column back into the 15 ml tube. Apply 3 ml of Buffer BW and centrifuge for 3 min at 2,000 xg (3,000 rpm).**
   Wipe off any spillage from the thread of the 15 ml tube before reinserting the Midi column.

8. **Discard the filtrate and re-insert the Midi column back into the 15 ml tube. Apply 3 ml of buffer TW, and centrifuge for 15 min at 4,500 xg (5,000 rpm).**
   If the column membrane has residual ethanol (originated from buffer TW) associated with it after centrifugation, incubate the Midi column for 15 min at room temperature to evaporate residual ethanol.
   This residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream reactions.

9. ***(Optional:)*** **If the centrifugal force applied at previous step is less than 4,500 xg, follow this;**

→ Discard the filtrate, wipe off any spillage from the thread of the 15 ml tube, and re-insert the Midi column back into the 15 ml tube. Apply 1 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the Midi column and incubate it at room temperature for 15 min.

Insufficient centrifugal force will bring on residual ethanol in Midi column membrane, followed by poor DNA recovery. At least, 4,000 xg is required for proper DNA recovery.

10. Place the Midi column into a new 15 ml centrifugation tube (provided). Pipet 300 ul of buffer AE or distilled water onto a center of membrane and close the cap. Incubate for 5 min at room temperature. Centrifuge for 5 min at 4,500 xg (5,000 rpm).

Before this elution step, it is strongly recommended that any residual ethanol originated from buffer TW should not remain in Midi column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions and etc.

Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the Midi column membrane for optimal elution of DNA.

Less than 300 ul of eluate will be obtained from 300 ul of elution buffer, but this has no influence on DNA yields.

If the volume of starting sample is less than 1 ml, less volume of buffer AE or distilled water can be applied. However, do not reduce the elution volume below 100 ul.

For long-term storage, eluting in buffer AE is recommended. But, EDTA included in the buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problems by using distilled water (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, make sure the pH of water is higher than 7.0.

11. **For higher concentrated yield, re-load the eluate from step 10 into the Midi column, close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 xg (5,000 rpm).**
Less than 300 ul of eluate will be obtained from 300 ul of elution buffer, but this has no influence on DNA yields.

For higher total yield, add 300 ul of fresh Buffer AE or distilled water again into the Midi column, close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 xg (5,000 rpm).
The first and second eluates can be combined or collected seperately as necessity.
Less than 300 ul of eluate will be obtained from 300 ul of elution buffer, but this has no influence on DNA yields.

B PROTOCOL for 1 ~ 2 ml of whole blood

Blood SV Midi

Clinic SV Midi

Before experiment

Prepare the water bath to 56°C

Prepare absolute ethanol

If a precipitate has formed in buffer BL, heat to dissolve completely

1. **Pipet 100 ul of Proteinase K solution (20 mg/ml, provided) into the bottom of a 15 ml centrifugation tube (not provided).**

2. **Add 2 ml of the sample to the tube and mix well.**
   If the sample volume is less than 2 ml, bring the volume of sample to 2 ml with PBS.

3. **Add 2.4 ml of Buffer BL to the tube. Vortex the tube for 15 sec to mix thoroughly.**
   For efficient lysis and consistent result, it is essential to mix the sample completely.

4. **Incubate at 56°C for 20 min.**
   During incubation, occasional vortexing of the lysate will help accelerate lysis. Longer incubation will not affect DNA recovery.

5. **Add 2 ml of absolute ethanol (not provided) to the sample, vortex to mix the sample thoroughly.**
   It is essential to mix the sample completely for efficient binding.

6. **Transfer 4 ml of the mixture to a Midi column carefully, close the cap, centrifuge for 3 min at 2,000 xg (3,000 rpm).**
   While transfer of the mixture to a Midi column, be careful not to moisten the rim of a Midi column.
   If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

7. **Discard the filtrate and re-insert the Midi column back into the 15 ml tube. Apply the remainder of the mixture, close the cap, and centrifuge for 3 min at 2,000 xg (3,000 rpm).**
   Wipe off any spillage from the thread of the 15 ml tube before reinserting the Midi column.
   While transfer of the mixture to the Midi column, be careful not to moisten the rim of Midi column.
   If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

8. **Discard the filtrate and re-insert the Midi column back into the 15 ml tube. Apply 3 ml of Buffer BW and centrifuge for 3 min at 2,000 xg (3,000 rpm).**
   Wipe off any spillage from the thread of the 15 ml tube before reinserting the Midi column.

9. **Discard the filtrate and re-insert the Midi column back into the 15 ml tube. Apply 3 ml of buffer TW, and centrifuge for 15 min at 4,500 xg (5,000 rpm).**
   If the column membrane has residual ethanol (originated from buffer TW) associated with it after centrifugation, incubate the Midi column for 15 min at room temperature to evaporate residual ethanol.
   This residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream reactions.

10. ***(Optional:)*** **If the centrifugal force applied at previous step is less than 4,500 xg, follow these;**

→ Discard the filtrate, wipe off any spillage from the thread of the 15 ml tube, and re-insert the Midi column back into the 15 ml tube. Apply 1 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the Midi column and incubate it at room temperature for 15 min.

Insufficient centrifugal force will bring on residual ethanol in Midi column membrane, followed by poor DNA recovery. At least, 4,000 xg is required for proper DNA recovery.

11. Place the Midi column into a new 15 ml centrifugation tube (provided). Pipet 400 ul of buffer AE or distilled water onto a center of membrane and close the cap. Incubate for 5 min at room temperature. Centrifuge for 5 min at 4,500 xg (5,000 rpm).

Before this elution step, it is strongly recommended that any residual ethanol originated from buffer TW should not remain in Midi column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions and etc.

Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the Midi column membrane for optimal elution of DNA.

Less than 400 ul of eluate will be obtained from 400 ul of elution buffer, but this has no influence on DNA yields.

If the volume of starting sample is less than 2 ml, less volume of buffer AE or distilled water can be applied. However, do not reduce the elution volume below 100 ul.

For long-term storage, eluting in buffer AE is recommended. But, EDTA included in the buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problems by using distilled water (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, make sure the pH of water is higher than 7.0.

12. For higher concentrated yield, re-load the eluate from step 11 into the Midi column, close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 xg (5,000 rpm).

Less than 400 ul of eluate will be obtained from 400 ul of elution buffer, but this has no influence on DNA yields.

For higher total yield, add 400 ul of fresh Buffer AE or distilled water again into the Midi column, close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 xg (5,000 rpm).

The first and second eluates can be combined or collected seperately as necessity.

Less than 400 ul of eluate will be obtained from 400 ul of elution buffer, but this has no influence on DNA yields.

C PROTOCOL for 3 ~ 5 ml of whole blood

Blood SV MAXI | Clinic SV MAXI | Cell SV MAXI

Before experiment

Prepare the water bath to 65°C

Prepare absolute ethanol

If a precipitate has formed in buffer BL, heat to dissolve completely

1. **Pipet 200 ul of Proteinase K solution (20 mg/ml, provided) into the bottom of a 50 ml conical tube (not provided).**

2. **Add 5 ml of the sample into the tube and mix well.**
   If the sample volume is less than 5 ml, bring the volume of sample to 5 ml with PBS.

3. **Add 6 ml of Buffer BL to the tube. Vortex the tube for 15 sec to mix thoroughly.**
   For efficient lysis and consistent result, it is essential to mix the sample completely and yield a homogenous mixture.

4. **Incubate at 65°C for 20 min.**
   During incubation, occasional vortexing of the lysate will help accelerate lysis. Longer incubation will not affect DNA recovery.

5. **Add 5 ml of absolute ethanol (not provided) to the sample, vortex to mix the sample thoroughly.**
   It is essential to mix the sample completely for efficient binding.

6. **Transfer all of the mixture to a MAXI column carefully, close the cap, centrifuge for 3 min at 2,000 xg (3,000 rpm).**
   While transfer of mixture to the MAXI column, be careful not to moisten the rim of MAXI column.
   Do NOT place the MAXI columns in tilted or bottom-up position even if caps are closed.
   If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

7. **Discard the filtrate and re-insert the MAXI column back into the 50 ml tube. Apply 7 ml of Buffer BW and centrifuge for 3 min at 2,000 xg (3,000 rpm).**
   Wipe off any spillage from the thread of the 50 ml tube before reinserting the MAXI column.

8. **Discard the filtrate, re-insert the MAXI column back into the 50 ml tube. Apply 10 ml of buffer TW, and centrifuge for 15 min at 4,500 xg (5,000 rpm).**
   If the MAXI column has residual ethanol (originated from buffer TW) associated with it after centrifugation, incubate the MAXI column for 15 min at room temperature to evaporate residual ethanol.
   This residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream reactions.

9. ***(Optional:)*** **If the centrifugal force applied at previous step is less than 4,500 xg, follow these;**

→ Discard the filtrate, wipe off any spillage from the thread of the 50 ml tube, and re-insert the MAXI column back into the 50 ml tube. Apply 3 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the MAXI column and incubate it for 20 min at room temperature.

Insufficient centrifugal force will bring on residual ethanol in MAXI column membrane, followed by poor DNA recovery. At least, 4,000 xg is required for proper DNA recovery.

10. Place the MAXI column into a new 50 ml centrifugation tube (provided). Pipet 600 ul of buffer AE or distilled water onto a center of membrane and close the cap. Incubate for 5 min at room temperature. Centrifuge at 4,500 xg (5,000 rpm) for 5 min.

Before this elution step, it is strongly recommended that any residual ethanol originated from buffer TW should not remain in MAXI column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions and etc.

Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the MAXI column membrane for optimal elution of DNA.

Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yields.

If the volume of starting sample is less than 5 ml, less volume of buffer AE or distilled water can be applied. However, do not reduce the elution volume below 300 ul.

For long-term storage, eluting in buffer AE is recommended. But, EDTA (0.5mM) included in the buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problem by using distilled water (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, make sure the pH of water (at least 7.0) before elution.

11. **For higher concentrated yield, re-load the eluate from step 10 into the MAXI column, close the cap, incubate 5 min at room temperature, and centrifuge at 4,500 xg (5,000 rpm) for 5 min.**
Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yield.

For higher total yield, add 600 ul of fresh buffer AE or distilled water again into the MAXI column, close the cap, incubate 5 min at room temperature, and centrifuge at 4,500 xg (5,000 rpm) for 5 min.
The first and second eluates can be combined or collected seperately as necessity.
Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yield.

D PROTOCOL for 6 ~ 10 ml of whole blood

** Due to the need of additional reagents, fewer preparations can be performed. Additional reagents can be purchased separately as accessory.*

Blood SV MAXI | Clinic SV MAXI | Cell SV MAXI

Before experiment

Prepare the water bath to 65°C

Prepare absolute ethanol

If a precipitate has formed in buffer BL, heat to dissolve completely

1. **Pipet 400 ul of Proteinase K solution (20 mg/ml, provided) into the bottom of a 50 ml conical tube (not provided).**

2. **Add 10 ml of the sample into the tube and mix well.**
   If the sample volume is less than 10 ml, bring the volume of sample to 10 ml with PBS.

3. **Add 12 ml of Buffer BL to the tube. Vortex the tube for 15 sec to mix thoroughly.**
   For efficient lysis and consistent result, it is essential to mix the sample completely and yield a homogenous mixture.

4. **Incubate at 65°C for 20 min.**
   During incubation, occasional vortexing of the lysate will help accelerate lysis. Longer incubation will not affect DNA recovery.

5. **Add 10 ml of absolute ethanol (not provided) to the sample, vortex to mix the sample thoroughly.**
   It is essential to mix the sample completely for efficient binding.

6. **Transfer a half of the mixture to a MAXI column carefully, close the cap, centrifuge for 3 min at 2,000 xg (3,000 rpm).**
   While transfer of mixture to the MAXI column, be careful not to moisten the rim of MAXI column.
   Do NOT place the MAXI columns in tilted or bottom-up position even if caps are closed.
   If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

7. **Discard the filtrate and re-insert the MAXI column back into the 50 ml tube. Apply the remainder of the mixture, close the cap, and centrifuge for 3 min at 2,000 xg (3,000 rpm)**
   Wipe off any spillage from the thread of the 50 ml tube before reinserting the MAXI column.
   While transfer of the mixture to the MAXI column, be careful not to moisten the rim of MAXI column.
   If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

8. **Discard the filtrate and re-insert the MAXI column back into the 50 ml tube. Apply 7 ml of Buffer BW and centrifuge for 3 min at 2,000 xg (3,000 rpm).**
   Wipe off any spillage from the thread of the 50 ml tube before reinserting the MAXI column.

9. **Discard the filtrate, re-insert the MAXI column back into the 50 ml tube. Apply 10 ml of buffer TW, and centrifuge for 15 min at 4,500 xg (5,000 rpm).**
   If the MAXI column has residual ethanol (originated from buffer TW) associated with it after centrifugation, incubate the MAXI column for 15 min at room temperature to evaporate residual ethanol.
   This residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream reactions.

10. ***(Optional:)*** **If the centrifugal force applied at previous step is less than 4,500 xg, follow these;**

→ Discard the filtrate, wipe off any spillage from the thread of the 50 ml tube, and re-insert the MAXI column back into the 50 ml tube. Apply 3 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the MAXI column and incubate it for 20 min at room temperature.

Insufficient centrifugal force will bring on residual ethanol in MAXI column membrane, followed by poor DNA recovery. At least, 4,000 xg is required for proper DNA recovery.

11. Place the MAXI column into a new 50 ml centrifugation tube (provided). Pipet 1 ml of buffer AE or distilled water onto a center of membrane and close the cap. Incubate for 5 min at room temperature. Centrifuge at 4,500 xg (5,000 rpm) for 5 min.

Before this elution step, it is strongly recommended that any residual ethanol originated from buffer TW should not remain in MAXI column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions and etc.

Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the MAXI column membrane for optimal elution of DNA.

Less than 1 ml of eluate will be obtained from 1 ml of elution buffer, but this has no influence on DNA yields.

If the volume of starting sample is less than 10 ml, less volume of buffer AE or distilled water can be applied. However, do not reduce the elution volume below 300 ul.

For long-term storage, eluting in buffer AE is recommended. But, EDTA (0.5mM) included in the buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problem by using distilled water (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, make sure the pH of water (at least 7.0) before elution.

12. **For higher concentrated yield, re-load the eluate from step 11 into the MAXI column, close the cap, incubate 5 min at room temperature, and centrifuge at 4,500 xg (5,000 rpm) for 5 min.**
Less than 1 ml of eluate will be obtained from 1 ml of elution buffer, but this has no influence on DNA yield.

For higher total yield, add 1 ml of fresh buffer AE or distilled water again into the MAXI column, close the cap, incubate 5 min at room temperature, and centrifuge at 4,500 xg (5,000 rpm) for 5 min.
The first and second eluates can be combined or collected seperately as necessity.
Less than 1 ml of eluate will be obtained from 1 ml of elution buffer, but this has no influence on DNA yield.

E PROTOCOL for ~ 2 x 10^7 of cultured cells

Clinic SV Midi

Before experiment

Prepare the water bath to 56°C and 70°C

Prepare absolute ethanol

If a precipitate has formed in buffer CL or BL, heat to dissolve completely

1. **Pellet cells (up to 2x10^7 cells) to a 15 ml microcentrifuge tube by centrifugation at 2,000 xg for 5 min.**
   Certain cell strains, such as PC12, are not lysed well in buffer CL. For those cells, it is helpful to perform additional freeze-thaw step several times before proceeding to next step.

2. **Discard the supernatant as much as possible and resuspend thoroughly cell pellet in 1 ml of Buffer CL.**
   Pelleted cells may not be resuspended easily in buffer CL. It is helpful to resuspend the cell pellet with residual media by flickering or vortexing before the addition of buffer CL.

3. **Add 100 ul of Proteinase K solution (20 mg/ml, provided). Mix thoroughly by vortexing. Incubate for 20 min at 56°C.**
   Vortex the lysate occasionally to accelerate during incubation.
   Longer incubation will not affect DNA recovery.

4. ***(Optional:)* If RNA-free DNA is required, cool the mixture to room temperature, add 20 ul of RNase solution (100 mg/ml, Cat.No.117-961), vortex to mix thoroughly, and incubate for 5 min at room temperature.**
   Unless RNase is treated, both DNA and RNA will be co-purified. RNA can inhibit some downstream enzymatic reactions, but not PCR itself.

5. **Add 1.2 ml of Buffer BL to the tube. Vortex the tube for 15 sec to mix thoroughly.**
   For efficient lysis and consistent result, it is essential to mix the sample completely.

6. **Incubate at 70°C for 10 min.**

7. **Add 1 ml of absolute ethanol (not provided) to the sample, vortex to mix the sample thoroughly.**
   It is essential to mix the sample completely for efficient binding.
   A white thread-like strands can be formed in the lysate. It is essential to transfer all of the lysate including this to a Midi column at next step.

8. **Transfer all of the mixture to a Midi column carefully, close the cap, centrifuge for 3 min at 2,000 xg (3,000 rpm).**
   While transfer of the mixture to a Midi column, be careful not to moisten the rim of a Midi column.
   If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

9. **Discard the filtrate and re-insert the Midi column back into the 15 ml tube. Apply 3 ml of Buffer BW and centrifuge for 3 min at 2,000 xg (3,000 rpm).**
   Wipe off any spillage from the thread of the 15 ml tube before reinserting the Midi column.

10. **Discard the filtrate and re-insert the Midi column back into the 15 ml tube. Apply 3 ml of buffer TW, and centrifuge for 15 min at 4,500 xg (5,000 rpm).**
    If the column membrane has residual ethanol (originated from buffer TW) associated with it after centrifugation, incubate the Midi column for 15 min at room temperature to evaporate residual ethanol.
    This residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream reactions.

11. ***(Optional:)*** **If the centrifugal force applied at previous step is less than 4,500 xg, follow this;**

→ Discard the filtrate, wipe off any spillage from the thread of the 15 ml tube, and re-insert the Midi column back into the 15 ml tube. Apply 1 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the Midi column and incubate it at room temperature for 15 min.

Insufficient centrifugal force will bring on residual ethanol in Midi column membrane, followed by poor DNA recovery. At least, 4,000 xg is required for proper DNA recovery.

12. Place the Midi column into a new 15 ml centrifugation tube (provided). Pipet 300 ul of buffer AE or distilled water onto a center of membrane and close the cap. Incubate for 5 min at room temperature. Centrifuge for 5 min at 4,500 xg (5,000 rpm).

Before this elution step, it is strongly recommended that any residual ethanol originated from buffer TW should not remain in Midi column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions and etc.

Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the Midi column membrane for optimal elution of DNA.

Less than 300 ul of eluate will be obtained from 300 ul of elution buffer, but this has no influence on DNA yields.

If the cell number of starting sample is small, less volume of buffer AE or distilled water can be applied. However, do not reduce the elution volume below 100 ul.

For long-term storage, eluting in buffer AE is recommended. But, EDTA included in the buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problems by using distilled water (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, make sure the pH of water is higher than 7.0.

13. **For higher concentrated yield, re-load the eluate from step 12 into the Midi column, close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 xg (5,000 rpm).**
Less than 300 ul of eluate will be obtained from 300 ul of elution buffer, but this has no influence on DNA yields.

For higher total yield, add 300 ul of fresh Buffer AE or distilled water again into the Midi column, close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 xg (5,000 rpm).
The first and second eluates can be combined or collected seperately as necessity.
Less than 300 ul of eluate will be obtained from 300 ul of elution buffer, but this has no influence on DNA yields.

F PROTOCOL for ~ 1 x 10^8 of cultured cells

Clinic SV MAXI | Cell SV MAXI

Before experiment

Prepare the water bath to 65°C and 70°C

Prepare absolute ethanol

If a precipitate has formed in buffer CL or BL, heat to dissolve completely

1. **Pellet cells (up to 1x10^8 cells) to a 50 ml microcentrifuge tube by centrifugation at 2,000 xg for 5 min.**
   Certain cell strains, such as PC12, are not lysed well in buffer CL. For those cells, it is helpful to perform additional freeze-thaw step several times before proceeding to next step.

2. **Discard the supernatant as much as possible and resuspend thoroughly cell pellet in 5 ml of Buffer CL.**
   Pelleted cells may not be resuspended easily in buffer CL. It is helpful to resuspend the cell pellet with residual media by flickering or vortexing before the addition of buffer CL.

3. **Add 200 ul of Proteinase K solution (20 mg/ml, provided). Mix thoroughly by vortexing. Incubate for 20 min at 65°C.**
   Vortex the lysate occasionally to accelerate during incubation.
   Longer incubation will not affect DNA recovery.

4. ***(Optional:)* If RNA-free DNA is required, cool the mixture to room temperature, add 100 ul of RNase solution (100 mg/ml, Cat. No.117-961), vortex to mix thoroughly, and incubate for 5 min at room temperature.**
   Unless RNase is treated, both DNA and RNA will be co-purified. RNA can inhibit some downstream enzymatic reactions, but not PCR itself.

5. **Add 6 ml of Buffer BL to the tube. Vortex the tube for 15 sec to mix thoroughly.**
   For efficient lysis and consistent result, it is essential to mix the sample completely and yield a homogenous mixture.

6. **Incubate at 70°C for 10 min.**

7. **Add 5 ml of absolute ethanol (not provided) to the sample, vortex to mix the sample thoroughly.**
   It is essential to mix the sample completely for efficient binding.
   A white thread-like strands can be formed in the lysate. It is essential to transfer all of the lysate including this to a Midi column at next step.

8. **Transfer all of the mixture to a MAXI column carefully, close the cap, centrifuge for 3 min at 2,000 xg (3,000 rpm).**
   While transfer of mixture to the MAXI column, be careful not to moisten the rim of MAXI column.
   Do NOT place the MAXI columns in tilted or bottom-up position even if caps are closed.
   If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

9. **Discard the filtrate and re-insert the MAXI column back into the 50 ml tube. Apply 7 ml of Buffer BW and centrifuge for 3 min at 2,000 xg (3,000 rpm).**
   Wipe off any spillage from the thread of the 50 ml tube before reinserting the MAXI column.

10. **Discard the filtrate, re-insert the MAXI column back into the 50 ml tube. Apply 10 ml of buffer TW, and centrifuge for 15 min at 4,500 xg (5,000 rpm).**
    If the MAXI column has residual ethanol (originated from buffer TW) associated with it after centrifugation, incubate the MAXI column for 15 min at room temperature to evaporate residual ethanol.
    This residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream reactions.

~ 1 x 10^8 Cultured cells

11. ***(Optional:)*** **If the centrifugal force applied at previous step is less than 4,500 xg, follow these;**

→ Discard the filtrate, wipe off any spillage from the thread of the 50 ml tube, and re-insert the MAXI column back into the 50 ml tube. Apply 3 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the MAXI column and incubate it for 20 min at room temperature.

Insufficient centrifugal force will bring on residual ethanol in MAXI column membrane, followed by poor DNA recovery. At least, 4,000 xg is required for proper DNA recovery.

12. Place the MAXI column into a new 50 ml centrifugation tube (provided). Pipet 600 ul of buffer AE or distilled water onto a center of membrane and close the cap. Incubate for 5 min at room temperature. Centrifuge at 4,500 xg (5,000 rpm) for 5 min.

Before this elution step, it is strongly recommended that any residual ethanol originated from buffer TW should not remain in MAXI column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions and etc.

Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the MAXI column membrane for optimal elution of DNA.

Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yields.

If the cell number of starting sample is small, less volume of buffer AE or distilled water can be applied. However, do not reduce the elution volume below 300 ul.

For long-term storage, eluting in buffer AE is recommended. But, EDTA (0.5mM) included in the buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problem by using distilled water (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, make sure the pH of water (at least 7.0) before elution.

13. **For higher concentrated yield, re-load the eluate from step 12 into the MAXI column, close the cap, incubate 5 min at room temperature, and centrifuge at 4,500 xg (5,000 rpm) for 5 min.**

Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yield.

For higher total yield, add 600 ul of fresh buffer AE or distilled water again into the MAXI column, close the cap, incubate 5 min at room temperature, and centrifuge at 4,500 xg (5,000 rpm) for 5 min.

The first and second eluates can be combined or collected seperately as necessity.

Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yield.

G PROTOCOL for 30 ~ 100 mg animal tissue

Clinic SV Midi

Before experiment

Prepare the water bath to 56°C and 70°C

Prepare absolute ethanol

If a precipitate has formed in buffer CL or BL, heat to dissolve completely

1. Homogenize 30 ~ 100 mg of tissue as described in step 1A, 1B or 1C, depending on the sample type.

It is most important to weigh the sample accurately.

If the sample is spleen tissue, up to 40 mg can be processed.

Well-disrupted sample will accelerate lysis and decrease the lysis time.

1A. For soft tissue, such as liver or brain, put up to 100 mg of the tissue into 15 ml conical tube, add 400 ul of Buffer CL, homogenize thoroughly on ice with homogenizer, add 600 ul of Buffer CL, and vortex vigorously to homogenate well.

1B. If a homogenizer is not available or the tissue is not soft, grind the tissue to a fine powder with liquid nitrogen in a pre-cooled mortar and pestle. Put up to 100 mg of the powdered tissue into 15 ml conical tube. Add 1 ml of Buffer CL and vortex for 30 sec to homogenate completely.

1C. If neither 1A nor 1B is available, mince the tissue with sterile sharp blade or scalpel as small as possible. Put up to 100 mg of the tissue into a 15 ml conical tube. Add 1 ml of Buffer CL and pulse-vortex for 30 sec.

** ***Alternatively, tissue sample can be effectively disrupted using some instruments, such as rotor-stator homogenizer or a bead-beater. When use these, follow the manufacture`s instruction manual.***

2. Add 100 ul of Proteinase K solution to the tube. Mix completely by vortexing or pipetting. Incubate at 56°C until the sample is completely lysed.

It is essential to mix the components completely for efficient lysis.

Lysis time varies from 10 min to 3 hr usually depending on the type of tissue and the homogenization method. The lysate should become translucent without any particles after complete lysis. Overnight lysis does not influence the preparation.

If the sample is lysed in water bath or heating block, vortex occasionally (2 ~ 3 times per hour) during incubation to lyse readily. *Lysis in shaking water bath, shaking incubator, or agitator would be best for efficient lysis.*

3. *(Optional:)* If RNA-free DNA is required, cool the lysate to room temperature, add 20 ul of RNase solution (100 mg/ml, Cat.No.117-961), vortex to mix thoroughly, and incubate for 3 min at room temperature.

Unless RNase is treated, both DNA and RNA will be co-purified. RNA can inhibit some downstream enzymatic reactions, but not PCR itself.

4. Add 1.2 ml of Buffer BL to the tube. Vortex the tube for 15 sec to mix thoroughly.

For efficient lysis and consistent result, it is essential to mix the sample completely.

5. Incubate at 70°C for 10 min.

6. Add 1 ml of absolute ethanol (not provided) to the sample, vortex to mix the sample thoroughly.

It is essential to mix the sample completely for efficient binding.

A white thread-like strands can be formed in the lysate. It is essential to transfer all of the lysate including this to a Midi column at next step.

7. Transfer all of the mixture to a Midi column carefully, close the cap, centrifuge for 3 min at 2,000 xg (3,000 rpm).

While transfer of the mixture to a Midi column, be careful not to moisten the rim of a Midi column.

If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

8. Discard the filtrate and re-insert the Midi column back into the 15 ml tube. Apply 3 ml of Buffer BW and centrifuge for 3 min at 2,000 xg (3,000 rpm).

Wipe off any spillage from the thread of the 15 ml tube before reinserting the Midi column.

9. Discard the filtrate and re-insert the Midi column back into the 15 ml tube. Apply 3 ml of buffer TW, and centrifuge for 15 min at 4,500 xg (5,000 rpm).

If the column membrane has residual ethanol (originated from buffer TW) associated with it after centrifugation, incubate the Midi column for 15 min at room temperature to evaporate residual ethanol.

This residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream reactions.

10. *(Optional:)* If the centrifugal force applied at previous step is less than 4,500 xg (5,000 rpm), follow this;

→ Discard the filtrate, wipe off any spillage from the thread of the 15 ml tube, and re-insert the Midi column back into the 15 ml tube. Apply 1 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the Midi column and incubate it at room temperature for 15 min.

Insufficient centrifugal force will bring on residual ethanol in Midi column membrane, followed by poor DNA recovery. At least, 4,000 xg is required for proper DNA recovery.

11. Place the Midi column into a new 15 ml centrifugation tube (provided). Pipet 300 ul of buffer AE or distilled water onto a center of membrane and close the cap. Incubate for 5 min at room temperature. Centrifuge for 5 min at 4,500 xg (5,000 rpm).

Before this elution step, it is strongly recommended that any residual ethanol originated from buffer TW should not remain in Midi column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions and etc.

Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the Midi column membrane for optimal elution of DNA.

Less than 300 ul of eluate will be obtained from 300 ul of elution buffer, but this has no influence on DNA yields.

If the weight or the cell number of starting sample is small, less volume of buffer AE or distilled water can be applied. However, do not reduce the elution volume below 100 ul.

For long-term storage, eluting in buffer AE is recommended. But, EDTA included in the buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problems by using distilled water (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, make sure the pH of water is higher than 7.0.

12. For higher concentrated yield, re-load the eluate from step 11 into the Midi column, close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 xg (5,000 rpm).

Less than 300 ul of eluate will be obtained from 300 ul of elution buffer, but this has no influence on DNA yields.

For higher total yield, add 300 ul of fresh Buffer AE or distilled water again into the Midi column, close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 xg (5,000 rpm)

The first and second eluates can be combined or collected seperately as necessity.

Less than 300 ul of eluate will be obtained from 300 ul of elution buffer, but this has no influence on DNA yields.

H PROTOCOL for 100 ~ 250 mg animal tissue

Clinic SV MAXI

Cell SV MAXI

Before experiment

Prepare the water bath to 56°C and 70°C

Prepare absolute ethanol

If a precipitate has formed in buffer CL or BL, heat to dissolve completely

1. Homogenize 100 ~ 250 mg of tissue as described in step 1A, 1B or 1C, depending on the sample type.

It is most important to weigh the sample accurately.

If the sample is spleen tissue, up to 100 mg can be processed.

Well-disrupted sample will accelerate lysis and decrease the lysis time.

1A. For soft tissue, such as liver or brain, put up to 250 mg of the tissue into homogenizer, add 1 ml of distilled water, homogenize thoroughly on ice. Transfer the homogenate into 50 ml conical tube, add 3 ml of Buffer CL, and vortex vigorously to homogenate well.

1B. If a homogenizer is not available or the tissue is not soft, grind the tissue to a fine powder with liquid nitrogen in a pre-cooled mortar and pestle. Put up to 250 mg of the powdered tissue into 50 ml conical tube. Add 4 ml of Buffer CL and vortex for 30 sec to homogenate completely.

1C. If neither 1A nor 1B is available, mince the tissue with sterile sharp blade or scalpel as small as possible. Put up to 250 mg of the tissue into a 50 ml conical tube. Add 4 ml of Buffer CL and pulse-vortex for 30 sec.

** ***Alternatively, tissue sample can be effectively disrupted using some instruments, such as rotor-stator homogenizer or a bead-beater. When use these, follow the manufacture`s instruction manual.***

2. Add 200 ul of Proteinase K solution to the tube. Mix completely by vortexing or pipetting. Incubate at 56°C until the sample is completely lysed.

It is essential to mix the components completely for efficient lysis.

Lysis time varies from 10 min to 3 hr usually depending on the type of tissue and the disruption method. The lysate should become translucent without any particles after complete lysis. Overnight lysis does not influence the preparation.

If the sample is lysed in water bath or heating block, vortex occasionally (2 ~ 3 times per hour) during incubation to lyse readily. *Lysis in shaking water bath, shaking incubator, or agitator would be best for efficient lysis.*

3. *(Optional:)* If RNA-free DNA is required, cool the lysate to room temperature, add 100 ul of RNase solution (100 mg/ml, Cat.No.117-961), vortex to mix thoroughly, and incubate for 3 min at room temperature.

Unless RNase is treated, both DNA and RNA will be co-purified. RNA can inhibit some downstream enzymatic reactions, but not PCR itself.

4. Add 5 ml of Buffer BL to the tube. Vortex the tube for 15 sec to mix thoroughly.

For efficient lysis and consistent result, it is essential to mix the sample completely.

5. Incubate at 70°C for 10 min.

6. Add 4 ml of absolute ethanol (not provided) to the sample, vortex to mix the sample thoroughly.

It is essential to mix the sample completely for efficient binding.

A white thread-like strands can be formed in the lysate. It is essential to transfer all of the lysate including this to a Midi column at next step.

7. Transfer all of the mixture to a MAXI column carefully, close the cap, centrifuge for 3 min at 2,000 xg (3,000 rpm).

While transfer of mixture to the MAXI column, be careful not to moisten the rim of MAXI column.

If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

Do NOT place the MAXI columns in tilted or bottom-up position even if caps are closed.

8. Discard the filtrate and re-insert the MAXI column back into the 50 ml tube. Apply 7 ml of Buffer BW and centrifuge for 3 min at 2,000 xg (3,000 rpm).

Wipe off any spillage from the thread of the 50 ml tube before reinserting the MAXI column.

9. Discard the filtrate, re-insert the MAXI column back into the 50 ml tube. Apply 10 ml of buffer TW, and centrifuge for 15 min at 4,500 xg (5,000 rpm).

If the MAXI column has residual ethanol (originated from buffer TW) associated with it after centrifugation, incubate the MAXI column for 15 min at room temperature to evaporate residual ethanol.

This residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream reactions.

10. *(Optional:)* If the centrifugal force applied at previous step is less than 4,500 xg, follow these;

→ Discard the filtrate, wipe off any spillage from the thread of the 50 ml tube, and re-insert the MAXI column back into the 50 ml tube. Apply 3 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the MAXI column and incubate it for 20 min at room temperature.

Insufficient centrifugal force will bring on residual ethanol in MAXI column membrane, followed by poor DNA recovery. At least, 4,000 xg is required for proper DNA recovery.

11. Place the MAXI column into a new 50 ml centrifugation tube (provided). Pipet 600 ul of buffer AE or distilled water onto a center of membrane and close the cap. Incubate for 5 min at room temperature. Centrifuge at 4,500 xg (5,000 rpm) for 5 min.

Before this elution step, it is strongly recommended that any residual ethanol originated from buffer TW should not remain in MAXI column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions and etc.

Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the MAXI column membrane for optimal elution of DNA.

Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yields.

If the weight or the cell number of starting sample is small, less volume of buffer AE or distilled water can be applied. However, do not reduce the elution volume below 300 ul.

For long-term storage, eluting in buffer AE is recommended. But, EDTA (0.5mM) included in the buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problem by using distilled water (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, make sure the pH of water (at least 7.0) before elution.

12. For higher concentrated yield, re-load the eluate from step 11 into the MAXI column, close the cap, incubate 5 min at room temperature, and centrifuge at 4,500 xg (5,000 rpm) for 5 min.

Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yield.

For higher total yield, add 600 ul of fresh buffer AE or distilled water again into the MAXI column, close the cap, incubate 5 min at room temperature, and centrifuge at 4,500 xg (5,000 rpm) for 5 min.

The first and second eluates can be combined or collected seperately as necessity.

Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yield.

I PROTOCOL for ~ 1 x 10^{10} gram (-) bacteria

Clinic SV Midi

Before experiment

Prepare the water bath to 56°C and 70°C

Prepare absolute ethanol

If a precipitate has formed in buffer CL or BL, heat to dissolve completely

1. **Harvest bacterial cells (up to 1 x 10^{10}) in a 15 ml conical tube by centrifugation at 10,000 xg for 5 min. Discard the supernatant as much as possible.**
   The number of cells in a bacterial culture varies depending on each strain. When A_{600}=1, 10 ml of bacterial culture may correspond to 1 ~ 2 x 10^{10} cells approximately.

2. **Resuspend the cell pellet with the residual liquid by flickering or vortexing.**

3. **Add 1 ml of buffer CL and resuspend completely by pipetting or vortexing.**

4. **Pipet 50 ul of Proteinase K solution (20 mg/ml, provided) and mix completely by pipetting or vortexing.**

5. **Incubate 56°C for 20 min.**
   Vortex the lysate occasionally during incubation to accelerate lysis.
   Longer incubation will not affect DNA recovery.

6. ***(Optional:)* If RNA-free DNA is required, cool the mixture to room temperature, add 20 ul of RNase solution (100 mg/ml, Cat.No.117-961), vortex to mix thoroughly, and incubate for 5 min at room temperature.**
   Unless RNase is treated, both DNA and RNA will be co-purified. RNA can inhibit some downstream enzymatic reactions, but not PCR itself.

7. Add 1.2 ml of Buffer BL to the tube. Vortex the tube for 15 sec to mix thoroughly.

For efficient lysis and consistent result, it is essential to mix the sample completely.

8. Incubate at 70°C for 10 min.

9. Add 1 ml of absolute ethanol (not provided) to the sample, vortex to mix the sample thoroughly.

It is essential to mix the sample completely for efficient binding.

A white thread-like strands can be formed in the lysate. It is essential to transfer all of the lysate including this to a Midi column at next step.

10. Transfer all of the mixture to a Midi column carefully, close the cap, centrifuge for 3 min at 2,000 xg (3,000 rpm).

While transfer of the mixture to a Midi column, be careful not to moisten the rim of a Midi column.

If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

11. Discard the filtrate and re-insert the Midi column back into the15 ml tube. Apply 3 ml of Buffer BW and centrifuge for 3 min at 2,000 xg (3,000 rpm).

Wipe off any spillage from the thread of the 15 ml tube before reinserting the Midi column.

12. Discard the filtrate and re-insert the Midi column back into the 15 ml tube. Apply 3 ml of buffer TW, and centrifuge for 15 min at 4,500 xg (5,000 rpm).

If the column membrane has residual ethanol(originated from buffer TW) associated with it after centrifugation, incubate the Midi column for 15 min at room temperature to evaporate residual ethanol.

This residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream reactions.

13. ***(Optional:)*** **If the centrifugal force applied at previous step is less than 4,500 x g, follow this;**

→ Discard the filtrate, wipe off any spillage from the thread of the 15 ml tube, and re-insert the Midi column back into the 15 ml tube. Apply 1 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the Midi column and incubate it at room temperature for 15 min.

Insufficient centrifugal force will bring on residual ethanol in Midi column membrane, followed by poor DNA recovery. At least, 4,000 xg is required for proper DNA recovery.

14. Place the Midi column into a new 15 ml centrifugation tube (provided). Pipet 300 ul of buffer AE or distilled water onto a center of membrane and close the cap. Incubate for 5 min at room temperature. Centrifuge for 5 min at 4,500 xg (5,000 rpm).

Before this elution step, it is strongly recommended that any residual ethanol originated from buffer TW should not remain in Midi column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions and etc.

Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the Midi column membrane for optimal elution of DNA.

Less than 300 ul of eluate will be obtained from 300 ul of elution buffer, but this has no influence on DNA yields.

If the cell number of starting sample is small, less volume of buffer AE or distilled water can be applied. However, do not reduce the elution volume below 100 ul.

For long-term storage, eluting in buffer AE is recommended. But, EDTA included in the buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problems by using distilled water (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, make sure the pH of water is higher than 7.0.

~ 1 x 10^{10} Gram (-) bacteria

15. **For higher concentrated yield, re-load the eluate from step 14 into the Midi column, close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 xg (5,000 rpm).**
Less than 300 ul of eluate will be obtained from 300 ul of elution buffer, but this has no influence on DNA yields.

For higher total yield, add 300 ul of fresh Buffer AE or distilled water again into the Midi column, close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 xg (5,000 rpm)
The first and second eluates can be combined or collected seperately as necessity.
Less than 300 ul of eluate will be obtained from 300 ul of elution buffer, but this has no influence on DNA yields.

J PROTOCOL for ~ 5 x 10^{10} gram (-) bacteria

Clinic SV MAXI | Cell SV MAXI

Before experiment

Prepare the water bath to 56°C and 70°C

Prepare absolute ethanol

If a precipitate has formed in buffer CL or BL, heat to dissolve completely

1. **Harvest bacterial cells (up to 5 x 10^{10}) in a 50 ml conical tube by centrifugation at 10,000 xg for 5 min. Discard the supernatant as much as possible.**
   The number of cells in a bacterial culture varies depending on each strain. When A_{600}=1, 10 ml of bacterial culture may correspond to 1 ~ 2 x 10^{10} cells approximately.

2. **Resuspend the cell pellet with the residual liquid by flickering or vortexing.**

3. **Add 5 ml of buffer CL and resuspend completely by pipetting or vortexing.**

4. **Pipet 200 ul of Proteinase K solution (20 mg/ml, provided) and mix completely by pipetting or vortexing.**

5. **Incubate 56°C for 30 min.**
   Vortex the lysate occasionally during incubation to accelerate lysis.
   Longer incubation will not affect DNA recovery.

6. ***(Optional:)* If RNA-free DNA is required, cool the mixture to room temperature, add 50 ul of RNase solution (100 mg/ml, Cat.No.117-961), vortex to mix thoroughly, and incubate for 5 min at room temperature.**
   Unless RNase is treated, both DNA and RNA will be co-purified. RNA can inhibit some downstream enzymatic reactions, but not PCR itself.

7. Add 6 ml of buffer BL to the tube. Vortex the tube to mix thoroughly.

8. Incubate at 70°C for 10 min.

9. Add 5 ml of absolute ethanol to the sample, mix thoroughly by inverting or pulse-vortexing.

It is essential to mix the sample completely for efficient binding.

A white thread-like strands can be formed in the lysate. It is essential to transfer all of the lysate including this to a Midi column at next step.

10. Transfer all of the mixture to a MAXI column carefully, close the cap, centrifuge for 3 min at 2,000xg (3,000 rpm).

While transfer of mixture to the MAXI column, be careful not to moisten the rim of MAXI column.

If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

Do NOT place the MAXI columns in tilted or bottom-up position even if caps are closed.

11. Discard the filtrate and re-insert the MAXI column back into the 50 ml tube. Apply 7 ml of Buffer BW and centrifuge for 2 min at 2,000 xg (3,000 rpm).

Wipe off any spillage from the thread of the 15 ml tube before reinserting the Midi column.

12. Discard the filtrate, re-insert the MAXI column back into the 50 ml tube. Apply 10 ml of buffer TW, and centrifuge for 15 min at 4,500 xg (5,000 rpm).

If the MAXI column has residual ethanol (originated from buffer TW) associated with it after centrifugation, incubate the MAXI column for 15 min at room temperature to evaporate residual ethanol.

This residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream reactions.

13. ***(Optional:)*** **If the centrifugal force applied at previous step is less than 4,500 xg, follow these;**

→ Discard the filtrate, wipe off any spillage from the thread of the 50 ml tube, and re-insert the MAXI column back into the 50 ml tube. Apply 3 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the MAXI column and incubate it for 20 min at room temperature.

Insufficient centrifugal force will bring on residual ethanol in MAXI column membrane, followed by poor DNA recovery. At least, 4,000 xg is required for proper DNA recovery.

14. Place the MAXI column into a new 50 ml centrifugation tube (provided). Pipet 600 ul of buffer AE or distilled water onto a center of membrane and close the cap. Incubate for 5 min at room temperature. Centrifuge at 4,500 xg (5,000 rpm) for 5 min.

Before this elution step, it is strongly recommended that any residual ethanol originated from buffer TW should not remain in MAXI column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions and etc.

Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the MAXI column membrane for optimal elution of DNA.

Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yields.

If the cell number of starting sample is small, less volume of buffer AE or distilled water can be applied. However, do not reduce the elution volume below 300 ul.

For long-term storage, eluting in buffer AE is recommended. But, EDTA (0.5mM) included in the buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problem by using distilled water (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, make sure the pH of water (at least 7.0) before elution.

~ 5 x 10^{10} Gram (-) bacteria

15. **For higher concentrated yield, re-load the eluate from step 14 into the MAXI column, close the cap, incubate 5 min at room temperature, and centrifuge at 4,500 xg (5,000 rpm) for 5 min.**
Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yield.

For higher total yield, add 600 ul of fresh buffer AE or distilled water again into the MAXI column, close the cap, incubate 5 min at room temperature, and centrifuge at 4,500 xg (5,000 rpm) for 5 min.
The first and second eluates can be combined or collected seperately as necessity.
Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yield.

K PROTOCOL for ~ 5 x 10^{10} gram (+) bacteria

Cell SV MAXI

Before experiment

Prepare the water bath to 37°C, 56°C and 70°C

Prepare Lysozyme or Lysostaphin

Prepare absolute ethanol

If a precipitate has formed in buffer CL or BL, heat to dissolve completely

All centrifugation should be performed at room temperature

Prepare Enzyme Mixture

Dissolve the appropriate enzyme (not provided, listed below) with buffer GP just before use. Enzyme mixture should be stored at -20°C (or below) as small aliquots; Ideally, once per an aliquot. Thawed aliquot should be discarded after use.

30 mg/mL lysozyme (LYS702, Bioshop, Canada or equivalents)
or/and
300 ug/mL lysostaphin (L7386, Sigma, USA or equivalents)

* For certain species, such as Staphylococcus, treatment of lysostaphin (final conc. = 300 ug/mL) may be required for efficient lysis instead of (or with) lysozyme. However, lysozyme is sufficient to lyse the cell wall for most gram positive bacterial strains.

1. **Harvest bacterial cells (up to 5 x 10^{10}) in a 50 ml conical tube by centrifugation at 10,000 xg for 5 min. Discard the supernatant as much as possible.**
   The number of cells in a bacterial culture varies depending on each strain. When A_{600}=1, 10 ml of bacterial culture may correspond to 1 ~ 2 x 10^{10} cells approximately.

2. **Resuspend the cell pellet with the residual liquid by flickering or vortexing.**

3. **Add 5 ml of the prepared enzyme mixture and resuspend completely by pipetting or vortexing.**

4. **Incubate at 37°C for 40 min.**
   The purpose of this treatment is to weaken the cell wall so that cell lysis can be efficiently taken place.

5. ***(Optional :)* If RNA-free DNA is required, add 50 ul of RNase solution (100 mg/ml, cat.no.117-961), vortex to mix thoroughly, and incubate for 5 min at room temperature.**
   Unless RNase A is treated, RNA will co-purified with DNA. RNA may inhibit some downstream enzymatic reactions, but not PCR itself.

6. **Add 6 ml of buffer BL and 200 ul of Proteinase K solution (20 mg/ml, provided) and vortex vigorously to mix completely.**

   Incubate 56°C for 30 min and then for a further 30 min at 70°C. Cool to room temperature.
   If pathogen, it is strongly recommended to substitute incubating at 70°C for 30 min by incubating at 95°C for 15 min.

8. Add 5 ml of absolute ethanol to the sample, mix thoroughly by inverting or pulse-vortexing.

It is essential to mix the sample completely for efficient binding.

A white thread-like strands can be formed in the lysate. It is essential to transfer all of the lysate including this to a Midi column at next step.

9. Transfer all of the mixture to a MAXI column carefully, close the cap, centrifuge for 3 min at 2,000xg (3,000 rpm).

While transfer of mixture to the MAXI column, be careful not to moisten the rim of MAXI column.

If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

Do NOT place the MAXI columns in tilted or bottom-up position even if caps are closed.

10. Discard the filtrate and re-insert the MAXI column back into the 50 ml tube. Apply 7 ml of buffer BW and centrifuge for 2 min at 2,000 xg (3,000 rpm).

Wipe off any spillage from the thread of the 15 ml tube before reinserting the Midi column

11. Discard the filtrate, re-insert the MAXI column back into the 50 ml tube. Apply 10 ml of buffer TW, and centrifuge for 15 min at 4,500 xg (5,000 rpm).

If the MAXI column has residual ethanol (originated from buffer TW) associated with it after centrifugation, incubate the MAXI column for 15 min at room temperature to evaporate residual ethanol.

This residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream reactions.

~ 5 x 10^{10} Gram (+) bacteria

12. ***(Optional:)*** **If the centrifugal force applied at previous step is less than 4,500 xg, follow these;**

→ Discard the filtrate, wipe off any spillage from the thread of the 50 ml tube, and re-insert the MAXI column back into the 50 ml tube. Apply 3 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the MAXI column and incubate it for 20 min at room temperature.

Insufficient centrifugal force will bring on residual ethanol in MAXI column membrane, followed by poor DNA recovery. At least, 4,000 xg is required for proper DNA recovery.

13. Place the MAXI column into a new 50 ml centrifugation tube (provided). Pipet 600 ul of buffer AE or distilled water onto a center of membrane and close the cap. Incubate for 5 min at room temperature. Centrifuge at 4,500 xg (5,000 rpm) for 5 min.

Before this elution step, it is strongly recommended that any residual ethanol originated from buffer TW should not remain in MAXI column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions and etc.

Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the MAXI column membrane for optimal elution of DNA.

Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yields.

If the cell number of starting sample is small, less volume of buffer AE or distilled water can be applied. However, do not reduce the elution volume below 300 ul.

For long-term storage, eluting in buffer AE is recommended. But, EDTA (0.5mM) included in the buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problem by using distilled water (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, make sure the pH of water (at least 7.0) before elution.

14. **For higher concentrated yield, re-load the eluate from step 13 into the MAXI column, close the cap, incubate 5 min at room temperature, and centrifuge at 4,500 xg (5,000 rpm) for 5 min.**
Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yield.

For higher total yield, add 600 ul of fresh buffer AE or distilled water again into the MAXI column, close the cap, incubate 5 min at room temperature, and centrifuge at 4,500 xg (5,000 rpm) for 5 min.
The first and second eluates can be combined or collected seperately as necessity.
Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yield.

~ 5 x 10^{10} Gram (+) bacteria

L PROTOCOL for ~ 5 x 10^8 yeast

Cell SV MAXI

Before experiment

Prepare the water bath to 37°C, 56°C and 70°C

Prepare Lyticase or Zymolase

Prepare absolute ethanol

1. **Add 30 ~ 50 ml (up to 5 x 10^8) of a yeast culture grown in YPD broth to a 50 ml conical tube.**
   When the value of A_{600} reaches to 1.0 (generally, log-phase), 30 ml of culture may yield 100 ~ 300 ug DNA approximately.

2. **Harvest cells in a 50 ml conical tube by centrifugation at 5,000 xg for 10 min. Discard the supernatant as much as possible.**

3. **Resuspend the cell pellet with the residual liquid by flickering or vortexing.**

4. **Add 5 ml of buffer YL and resuspend completely by pipetting or vortexing.**

5. **Add 2,000 U of lyticase or 200 U of zymolase and gently pipet to mix completely.**
   Unit/mg of enzyme will vary depending on the manufactures.

6. **Incubate at 37°C for 1 hr to digest the cell wall.**
   Incubated cells turn to spheroplasts at this step, and this makes easy the followed lysis step.

7. **Centrifuge at 5,000 xg for 10 min and remove the supernatant as much as possible.**

8. **Resuspend the cell pellet with the residual liquid by flickering or vortexing.**

9. **Add 5 ml of buffer CL and resuspend completely by pipetting or vortexing.**

10. **Pipet 200 ul of Proteinase K solution (20 mg/ml, provided) and mix completely by pipetting or vortexing.**

11. **Incubate 56°C for 30 min.**
    Vortex the lysate occasionally during incubation to accelerate lysis.
    Longer incubation will not affect DNA recovery.

12. ***(Optional:)*** **If RNA-free DNA is required, cool the mixture to room temperature, add 50 ul of RNase solution (100 mg/ml, Cat.No.117-961), vortex to mix thoroughly, and incubate for 5 min at room temperature.**
    Unless RNase is treated, both DNA and RNA will be co-purified. RNA can inhibit some downstream enzymatic reactions, but not PCR itself.

13. **Add 6 ml of buffer BL to the tube. Vortex the tube to mix thoroughly.**

14. **Incubate at 70°C for 10 min.**

15. **Add 5 ml of absolute ethanol to the sample, mix thoroughly by inverting or pulse-vortexing.**
    It is essential to mix the sample completely for efficient binding.
    A white thread-like strands can be formed in the lysate. It is essential to transfer all of the lysate including this to a Midi column at next step.

~ 5×10^8 Yeast

16. Transfer all of the mixture to a MAXI column carefully, close the cap, centrifuge for 3 min at 2,000xg (3,000 rpm).

While transfer of mixture to the MAXI column, be careful not to moisten the rim of MAXI column.

If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

Do NOT place the MAXI columns in tilted or bottom-up position even if caps are closed.

17. Discard the filtrate and re-insert the MAXI column back into the 50 ml tube. Apply 7 ml of Buffer BW and centrifuge for 2 min at 2,000 xg (3,000 rpm).

Wipe off any spillage from the thread of the 15 ml tube before reinserting the Midi column.

18. Discard the filtrate, re-insert the MAXI column back into the 50 ml tube. Apply 10 ml of buffer TW, and centrifuge for 15 min at 4,500 xg (5,000 rpm).

If the MAXI column has residual ethanol (originated from buffer TW) associated with it after centrifugation, incubate the MAXI column for 15 min at room temperature to evaporate residual ethanol.

This residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream reactions.

19. ***(Optional:)*** **If the centrifugal force applied at previous step is less than 4,500 xg, follow these;**

→ Discard the filtrate, wipe off any spillage from the thread of the 50 ml tube, and re-insert the MAXI column back into the 50 ml tube. Apply 3 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the MAXI column and incubate it for 20 min at room temperature.

Insufficient centrifugal force will bring on residual ethanol in MAXI column membrane, followed by poor DNA recovery. At least, 4,000 xg is required for proper DNA recovery.

20. Place the MAXI column into a new 50 ml centrifugation tube (provided). Pipet 600 ul of buffer AE or distilled water onto a center of membrane and close the cap. Incubate for 5 min at room temperature. Centrifuge at 4,500 xg (5,000 rpm) for 5 min.

Before this elution step, it is strongly recommended that any residual ethanol originated from buffer TW should not remain in MAXI column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions and etc.

Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the MAXI column membrane for optimal elution of DNA.

Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yields.

If the cell number of starting sample is small, less volume of buffer AE or distilled water can be applied. However, do not reduce the elution volume below 300 ul.

For long-term storage, eluting in buffer AE is recommended. But, EDTA (0.5mM) included in the buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problem by using distilled water (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, make sure the pH of water (at least 7.0) before elution.

~ 5 x 10^8 Yeast

21. **For higher concentrated yield, re-load the eluate from step 20 into the MAXI column, close the cap, incubate 5 min at room temperature, and centrifuge at 4,500 xg (5,000 rpm) for 5 min.**
Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yield.

For higher total yield, add 600 ul of fresh buffer AE or distilled water again into the MAXI column, close the cap, incubate 5 min at room temperature, and centrifuge at 4,500 xg (5,000 rpm) for 5 min.
The first and second eluates can be combined or collected seperately as necessity.
Less than 600 ul of eluate will be obtained from 600 ul of elution buffer, but this has no influence on DNA yield.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low or no recovery	**Low cells in the sample**	Some blood may have low concentration of white blood cells. Increase the sample volumes and load the column for binding several times. Reduce the elution volume to minimum. If possible, draw new blood sample and repeat the DNA purification with new sample.
	Too much starting sample	If too much cells present in the sample, reduce the starting sample weight or volume, or increase the volume of buffers to double.
	Starting material was too old or mis-stored.	Best result is obtained when using a fresh sample. DNA yield is dependent on the type, size, age and storage condition of starting material. Lower yield may be obtained from the material that has not been appropriately stored. If blood, samples that have been stored at 4°C for more than 5 days may give reduced yield.
	Inefficient lysis	Inefficient lysis may be due to insufficient mixing with Buffer BL, too much cells in the starting sample, or degenerated Proteinase K. After addition of Buffer CL or BL in procedures, vortex the mixture vigorously and immediately to mix completely.
	Weaken activity of Proteinase K caused by mis-storage or out-of-date	Proteinase K should be stored at 4°C for maintenance of proper activity. However, it is recommended to store at -20°C for prolonged preservation of its activity. Lysis can not be done properly with degenerated Proteinase K.

Facts	Possible Causes	Suggestions
Low or no recovery	**The centrifugation step in procedure is performed on the fixed-angle centrifuge.**	The centrifugation step in procedure must be carried out on swinging-bucket-have centrifuge. If not, DNA yields may be significantly reduced.
	G-force was not reach to 4,500 x g	For proper DNA purification, centrifugal g-force should be reached to 4,500 x g at least. If g-force is under 4,500 x g, refer to the step 11.
	Column not incubated for 5 min	After addition of Buffer AE or distilled water, the column should be incubated at room temperature for 5 min before centrifugation.
	Improper eluent	As user's requirement, elution buffer other than Buffer AE can be used. However, the optimal conditions for elution should be considered carefully whether low salt concentration with alkaline pH (7<pH<9). When water or other buffer was employed as eluent, ensure that conditions.
SV column has colored residue associated with it after wash, resulting in colored residue	**Inefficient lysis**	Inefficient lysis may cause that colored residue remains on the column membrane. Repeat the procedure after consideration of 'Inefficient lysis' at 'Low or no recovery'.
	Incomplete removal of hemoglobin	In case of DNA preparation from certain animal blood, it is hard to remove hemoglobin from their blood. Carry out additional wash step with Buffer BW.

Facts	Possible Causes	Suggestions
Low $A_{260/280}$ ratio	**Insufficient lysis**	Insufficient lysis cause low DNA purity, and is due to incomplete mixing with Buffer BL or ethanol, too much cells in the starting sample, or degenerated Proteinase K. Check these out in next preparations.
	Residual ethanol from Buffer TW remains in eluate	If centrifugation has been performed on fixed-angle rotor or centrifugal force does not reach 4,000 xg, residual ethanol will remain in the column membrane and it causes low purity. To remove residual ethanol completely, refer to the optional step.
	Incomplete removal of hemoglobin	In case of DNA preparation from certain animal blood, it is hard to remove hemoglobin from their blood. Carry out additional wash step with Buffer BW.
High $A_{260/280}$ ratio	**RNA contamination**	RNA may inhibit some downstream enzymatic reactions. If RNA-free DNA is required, carry out the optional step.
Low concentration of DNA in eluate	**Low cells in starting sample (too high elution volume)**	Increase the volume of starting sample, or reduce the elution volume to minimum or do re-elution with eluate. Check the last step in procedures for higher concentrations of DNA.
Column clogging	**Inefficient lysis**	Inefficient lysis may lead to column clogging. About inefficient lysis, check 'Inefficient lysis' at 'Low or no recovery'.
Degraded DNA	**Starting sample is too old or mis-stored**	Too old or mis-stored sample often yield degraded DNA. Use fresh sample.

Facts	Possible Causes	Suggestions
DNA floats out of well while loading of agarose gel	**Residual ethanol from Buffer TW remains in eluate**	If centrifugation has been performed on fixed-angle rotor or centrifugal force does not reach 4,500 xg, residual ethanol will remain in the column membrane. Residual ethanol interferes with DNA in sinking into a well because of its low specific gravity. To remove residual ethanol completely, refer to the optional step.
Enzymatic reaction is not performed well with purified DNA	**Low purity of DNA**	Check 'Low $A_{260/280}$ ratio'
	RNA contamination	RNA may inhibit some downstream enzymatic reactions. If RNA-free DNA is required, carry out the optional step.
	Incomplete removal of hemoglobin	In case of DNA preparation from certain animal blood, it is hard to remove hemoglobin from their blood. Carry out additional wash step with Buffer BW.
	High salt concentration in eluate	Ensure that all washing steps were performed just in accordance with the protocols. Optionally, additional washing with buffer TW can help remove some more salts.
Precipitate in some buffer	**Buffer stored in cool ambient condition**	For proper DNA purification, any precipitate in a Buffer should be dissolved by incubating it at 37°C (or above) until it disappears.

Ordering Information

GeneAll® *Hybrid-Q™* *for rapid preparation of plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid Rapidprep	mini	50	100-150	spin
		200	100-102	

GeneAll® *Exprep™* *for preparation of plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid SV	mini	50	101-150	spin / vacuum
		200	101-102	
		1,000	101-111	
	Midi	26	101-226	spin / vacuum
		50	101-250	
		100	101-201	

GeneAll® *Exfection™*
for preparation of transfection-grade plasmid DNA

Products	Scale	Size	Cat. No.	Type
Plasmid LE *(Low Endotoxin)*	mini	50	111-150	spin / vacuum
		200	111-102	
	Midi	26	111-226	spin / vacuum
		100	111-201	
Plasmid EF *(Endotoxin Free)*	Midi	20	121-220	spin
		100	121-201	

GeneAll® *Expin™* *for purification of fragment DNA*

Products	Scale	Size	Cat. No.	Type
Gel SV	mini	50	102-150	spin / vacuum
		200	102-102	
PCR SV	mini	50	103-150	spin / vacuum
		200	103-102	
CleanUp SV	mini	50	113-150	spin / vacuum
		200	113-102	
Combo GP	mini	50	112-150	spin / vacuum
		200	112-102	

GeneAll® *Exgene™* *for isolation of total DNA*

Products	Scale	Size	Cat. No.	Type
Tissue SV	mini	100	104-101	spin / vacuum
		250	104-152	
	Midi	26	104-226	spin / vacuum
		100	104-201	
	MAXI	10	104-310	spin / vacuum
		26	104-326	
Tissue *plus!* SV	mini	100	109-101	spin / vacuum
		250	109-152	
	Midi	26	109-226	spin / vacuum
		100	109-201	
	MAXI	10	109-310	spin / vacuum
		26	109-326	

GeneAll® *Exgene™* *for isolation of total DNA*

Products	Scale	Size	Cat. No.	Type
Blood SV	mini	100	105-101	spin / vacuum
		250	105-152	
	Midi	26	105-226	spin / vacuum
		100	105-201	
	MAXI	10	105-310	spin / vacuum
		26	105-326	
Cell SV	mini	100	106-101	spin / vacuum
		250	106-152	
	MAXI	10	106-310	spin / vacuum
		26	106-326	
Clinic SV	mini	100	108-101	spin / vacuum
		250	108-152	
	Midi	26	108-226	spin / vacuum
		100	108-201	
	MAXI	10	108-310	spin / vacuum
		26	108-326	
Genomic DNA micro		50	118-050	spin
Plant SV	mini	100	117-101	spin / vacuum
		250	117-152	
	Midi	26	117-226	spin / vacuum
		100	117-201	
	MAXI	10	117-310	spin / vacuum
		26	117-326	
Soil DNA mini	mini	50	114-150	spin
Stool DNA mini	mini	50	115-150	spin
Viral DNA / RNA	mini	50	128-150	spin

GeneAll® *GenEx™* *for isolation of total DNA without spin column*

Products	Scale	Size	Cat. No.	Type
GenEx™ Blood	Sx	100	220-101	solution
		500	220-105	
	Lx	100	220-301	solution
GenEx™ Cell	Sx	100	221-101	solution
		500	221-105	
	Lx	100	221-301	solution
GenEx™ Tissue	Sx	100	222-101	solution
		500	222-105	
	Lx	100	222-301	solution

Products	Scale	Size	Cat. No.	Type
GeneAll® *GenEx™* *for isolation of total DNA*				
GenEx™ Plant	Sx	100	227-101	solution
	Mx	100	227-201	
	Lx	100	227-301	
GenEx™ Plant *plus!*	Sx	100	228-101	solution
	Mx	50	228-250	
	Lx	20	228-320	
GeneAll® *DirEx™* series *for preperation of PCR-template without extraction*				
DirEx™		100	250-101	solution
DirEx™ *Fast*-Tissue		96 T	260-011	solution
DirEx™ *Fast*-Cultured cell		96 T	260-021	solution
DirEx™ *Fast*-Whole blood		96 T	260-031	solution
DirEx™ *Fast*-Blood stain		96 T	260-041	solution
DirEx™ *Fast*-Hair		96 T	260-051	solution
DirEx™ *Fast*-Buccal swab		96 T	260-061	solution
DirEx™ *Fast*-Cigarette		96 T	260-071	solution
GeneAll® RNA series *for preperation of total RNA*				
RiboEx™	mini	100	301-001	solution
		200	301-002	
Hybrid-R™	mini	100	305-101	spin
Hybrid-R™ Blood RNA	mini	50	315-150	spin
Hybrid-R™ miRNA	mini	50	325-150	spin
RiboEx™ LS	mini	100	302-001	solution
		200	302-002	
Riboclear™	mini	50	303-150	spin
Riboclear™ *plus!*	mini	50	313-150	spin
Ribospin™	mini	50	304-150	spin
Ribospin ™ vRD	mini	50	302-150	spin
Ribospin ™ vRD *plus!*	mini	50	312-150	spin
Ribospin ™ vRD II	mini	50	322-150	spin
Ribospin ™ Plant	mini	50	307-150	spin
Ribospin™ Seed / Fruit	mini	50	317-150	spin
Allspin™	mini	50	306-150	spin
RiboSaver™	mini	100	351- 001	solution

Products	Scale	Size	Cat. No.	Type
GeneAll® *AmpONE™* *for PCR amplification*				
Taq DNA polymerase		250 U	501-025	(2.5 U/μℓ)
		500 U	501-050	
		1,000 U	501-100	
α-Taq DNA polymerase		250 U	502-025	(2.5 U/μℓ)
		500 U	502-050	
		1,000 U	502-100	
α-Pfu DNA polymerase		250 U	504-025	(2.5 U/μℓ)
		500 U	504-050	
		1,000 U	504-100	
Fast-Pfu DNA polymerase		250 U	505-025	(2.5 U/μℓ)
		500 U	505-050	
		1,000 U	505-100	
Hotstart Taq DNA polymerase		250 U	531-025	(2.5 U/μℓ)
		500 U	531-050	
		1,000 U	531-100	
Taq Premix	96 tubes	20 μℓ	521-200	lyophilized
		50 μℓ	521-500	
		20 μℓ	526-200	solution
		50 μℓ	526-500	
α-Taq Premix	96 tubes	20 μℓ	522-200	lyophilized
		50 μℓ	522-500	
		20 μℓ	527-200	solution
		50 μℓ	527-500	
HS-Taq Premix	96 tubes	20 μℓ	525-200	solution
		50 μℓ	525-500	
		20 μℓ	520-200	lyophilized
α-Pfu Premix	96 tubes	50 μℓ	523-500	solution
Taq Premix (w/o dye)	96 tubes	20 μℓ	524-200	lyophilized
dNTPs mix		500 μℓ	509-020	2.5 mM each
dNTPs set (set of dATP, dCTP, dGTP and dTTP)		1 ml x 4 tubes	509-040	100 mM

* Each dNTPs is available

Products	Scale	Size	Cat. No.	Type
GeneAll® *AmpMaster™* *for PCR amplification*				
Taq Master mix	0.5 ml x 2 tubes		541-010	solution
	0.5 ml x 10 tubes		541-050	solution
α-Taq Master mix	0.5 ml x 2 tubes		542-010	solution
	0.5 ml x 10 tubes		542-050	solution
HS-Taq Master mix	0.5 ml x 2 tubes		545-010	solution
	0.5 ml x 10 tubes		545-050	solution
α-Pfu Master mix	0.5 ml x 2 tubes		543-010	solution
	0.5 ml x 10 tubes		543-050	solution
GeneAll® *HyperScript™* *for Reverse Transcription*				
Reverse Transcriptase	10,000 U		601-100	solution
RT Master mix	0.5 ml × 2 tubes		601-710	solution
RT Master mix with oligo $(dT)_{20}$	0.5 ml × 2 tubes		601-730	solution
RT Master mix with random hexamer	0.5 ml × 2 tubes		601-740	solution
RT Premix	96 tubes, 20 μl		601-602	solution
RT Premix with oligo $(dT)_{20}$	96 tubes, 20 μl		601-632	solution
RT Premix with random hexamer	96 tubes, 20 μl		601-642	solution
One-step RT-PCR Master mix	0.5 ml × 2 tubes		602-110	solution
One-step RT-PCR Premix	96 tubes, 20 μl		602-102	solution
First strand Synthesis Kit	50 reaction		605-005	solution
ZymAll™ RNase Inhibitor	10,000 U		605-010	solution
ZymAll™ RNase Inhibitor	4,000 U		605-004	solution
GeneAll® Protein series				
ProtinEx™ Animal cell / tissue	100 ml		701-001	solution
PAGESTA™ Reducing 5X SDS-PAGE Sample Buffer	1 ml × 10 tubes		751-001	solution

Products	Size	Cat. No.
GeneAll® STEADi™ *for automatic nucleic acid purification*		
STEADi™ 12 Instrument		GST012
STEADi™ 24 Instrument		GST024
STEADi™ Genomic DNA Cell / Tissue Kit	96	401-104
STEADi™ Genomic DNA Blood Kit	96	402-105
STEADi™ Bacteria DNA Kit	96	403-106
STEADi™ Total RNA Kit	96	404-304
STEADi™ Viral DNA/RNA Kit	96	405-322
STEADi™ CFC Seed DNA/RNA Kit	96	406-C02

Note

GeneAll

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2010.05